

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Nan, Guo-Ling
Nagai, Chifumi
- (ii) TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR GENETIC
TRANSFORMATION OF PINEAPPLE
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Medlen & Carroll, LLP
 - (B) STREET: 220 Montgomery Street, Suite 2200
 - (C) CITY: San Francisco
 - (D) STATE: California
 - (E) COUNTRY: United States of America
 - (F) ZIP: 94104
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 09/078,862
 - (B) FILING DATE: 14-MAY-1998
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Carroll, Peter G.
 - (B) REGISTRATION NUMBER: 32,837
 - (C) REFERENCE/DOCKET NUMBER: UH-03321
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (415) 705-8410
 - (B) TELEFAX: (415) 397-8338

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2145 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3509 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "DNA"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 9 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "DNA"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 623 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: circular

- (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "DNA"

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 16 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: not relevant
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met	Ala	Asp	Ala	Glu	Asp	Gly	Gly	Ser	Pro	Gly	Gly	Gln	Ser	Phe	Met
1				5				10					15		

CLAIMS

1. A stably transformed transgenic pineapple-like totipotent body.
2. The stably transformed transgenic pineapple-like totipotent body of Claim 1, wherein said totipotent body is a protocorm-like body.
3. The stably transformed transgenic pineapple-like totipotent body of Claim 1, wherein said totipotent body is a callus.
4. The stably transformed transgenic pineapple-like totipotent body of Claim 1, wherein said totipotent body comprises undifferentiated cells.
5. The stably transformed transgenic pineapple-like totipotent body of Claim 1, wherein said totipotent body is a pineapple totipotent body.
6. A stably transformed transgenic pineapple-like plant.
7. The stably transformed transgenic pineapple-like plant of Claim 6, wherein said plant is derived from a transgenic pineapple-like totipotent body.
8. The stably transformed transgenic pineapple-like plant of Claim 7, wherein said totipotent body is a protocorm-like body.
9. The stably transformed transgenic pineapple-like plant of Claim 7, wherein said totipotent body is a callus.
10. The stably transformed transgenic pineapple-like plant of Claim 6, wherein said plant is a pineapple plant.

11. A method for producing a stably transformed transgenic pineapple-like totipotent body, comprising:
- a) providing:
 - i) a pineapple-like totipotent body; and
 - ii) a heterologous nucleic acid comprising an oligonucleotide sequence of interest; and
 - b) introducing said oligonucleotide sequence of interest into said pineapple-like totipotent body under conditions such that a stably transformed transgenic pineapple-like totipotent body is produced.
12. The method of Claim 11, wherein said pineapple-like totipotent body is a callus.
13. The method of Claim 11, wherein said pineapple-like totipotent body is a protocorm-like body.
14. The method of Claim 11, further comprising c) selecting said transgenic pineapple-like totipotent body.
15. The method of Claim 14, wherein said selecting is in liquid medium.
16. The method of Claim 14, wherein said selecting comprises detecting said oligonucleotide in the genome of said stable transformed pineapple-like totipotent body.
17. The method of Claim 11, wherein said pineapple-like totipotent body is a pineapple totipotent body.
18. The method of Claim 11, wherein said oligonucleotide is introduced by bombarding said pineapple-like totipotent body with said oligonucleotide sequence of interest.
19. The method of Claim 11, wherein said oligonucleotide is introduced by infecting said pineapple-like totipotent body with *Agrobacterium* comprising said oligonucleotide sequence of interest.

20. The method of Claim 19, wherein said infecting comprises microwounding said pineapple-like totipotent body to produce a microwounded totipotent body, and infecting said microwounded totipotent body with said *Agrobacterium*.
21. The method of Claim 19, wherein said *Agrobacterium* is agropine-type.
22. The method of Claim 19, wherein said *Agrobacterium* is nopaline-type.
23. The method of Claim 19, wherein said *Agrobacterium* is octopine-type.
24. A method for producing a stably transformed transgenic pineapple-like plant, comprising:
- a) providing:
 - i) a pineapple-like totipotent body; and
 - ii) a heterologous nucleic acid comprising an oligonucleotide sequence of interest;
 - b) introducing said oligonucleotide sequence of interest into said pineapple-like totipotent body under conditions such that a transgenic pineapple-like totipotent body is produced; and
 - c) culturing said transgenic pineapple-like totipotent body under conditions such that a stably transformed transgenic pineapple-like plant is produced.
25. The method of claim 24, wherein said oligonucleotide is introduced by bombarding said pineapple-like totipotent body with said oligonucleotide sequence of interest.
26. The method of Claim 24, wherein said oligonucleotide is introduced by infecting said pineapple-like totipotent body with *Agrobacterium* comprising said oligonucleotide sequence of interest.
27. The method of Claim 24, further comprising prior to step c) selecting said stably transformed transgenic pineapple-like totipotent body.
28. The method of Claim 27, wherein said selecting is in liquid medium.

29. The method of Claim 27, wherein said selecting comprises detecting said oligonucleotide in the genome of said stably transformed transgenic pineapple-like totipotent body.
30. The method of Claim 24, wherein said pineapple-like plant is a pineapple plant.
31. The method of Claim 24, wherein said sequence of interest is selected from the group consisting of oligonucleotides encoding sucrose phosphate synthase, CpTi, thaumatin, and ACC deaminase.
32. The method of Claim 24, wherein said sequence of interest is selected from the group consisting of antisense polyphenol oxidase and ACC oxidase.
33. A method for producing a pineapple-like protocorm-like body comprising maintaining said pineapple-like protocorm-like body in liquid medium.
34. The method of Claim 33, wherein said liquid medium substantially comprises PI medium.
35. The method of Claim 33, wherein said pineapple-like protocorm-like body is a pineapple protocorm-like body.

Figure 1

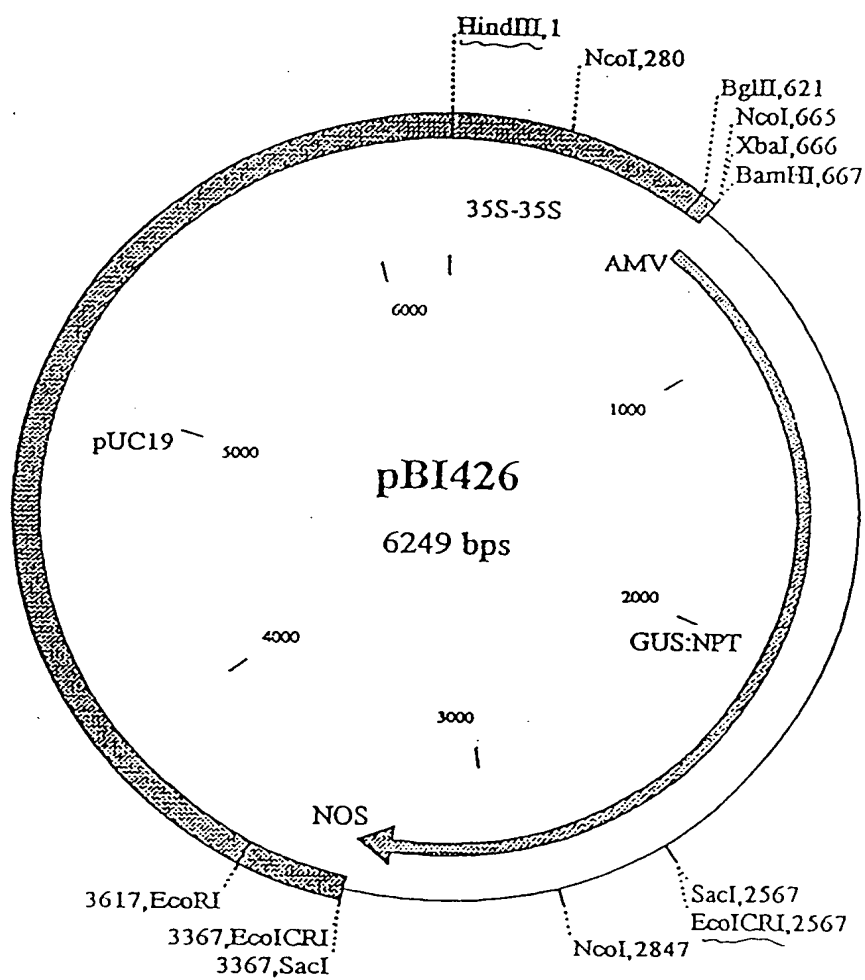


Figure 4

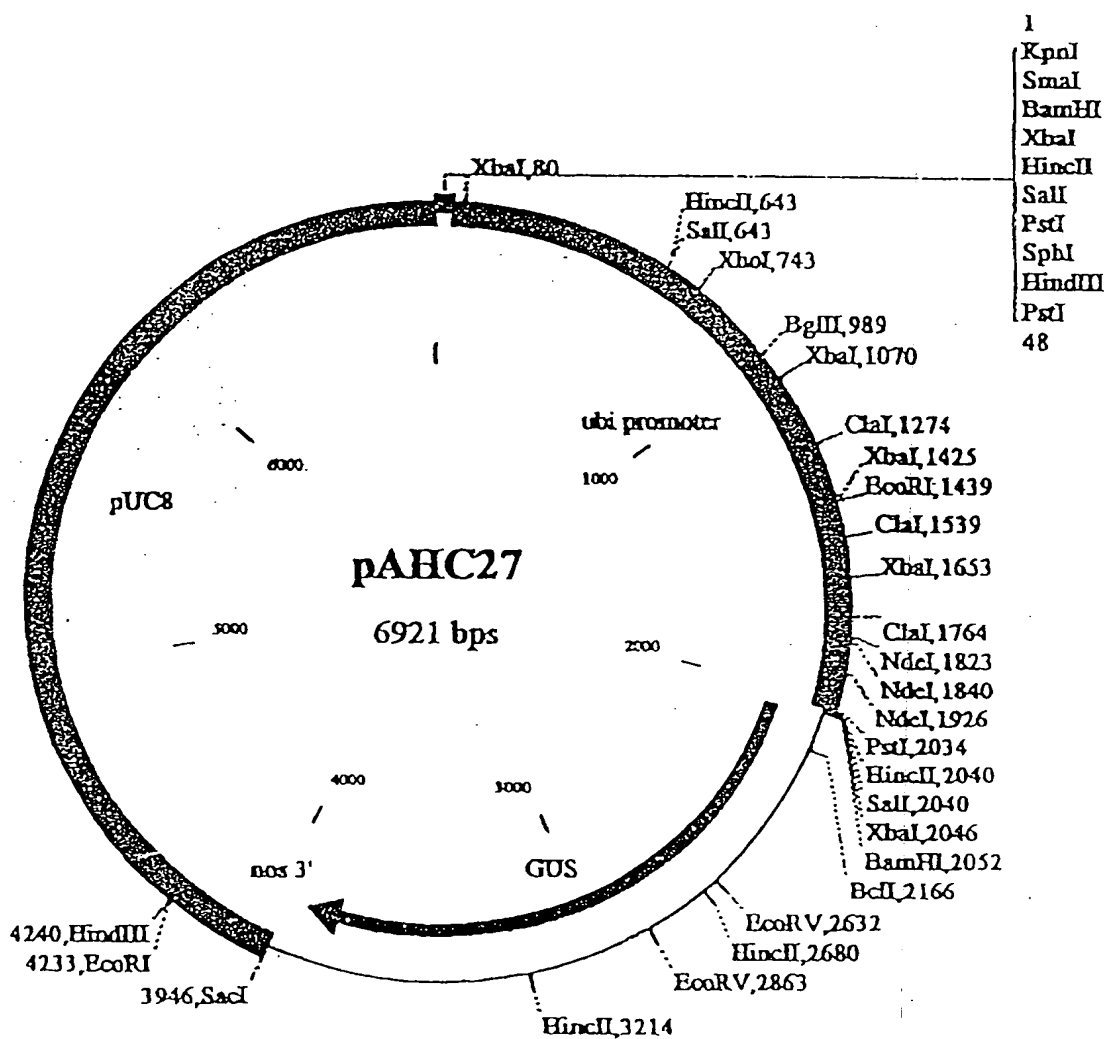


Figure 5

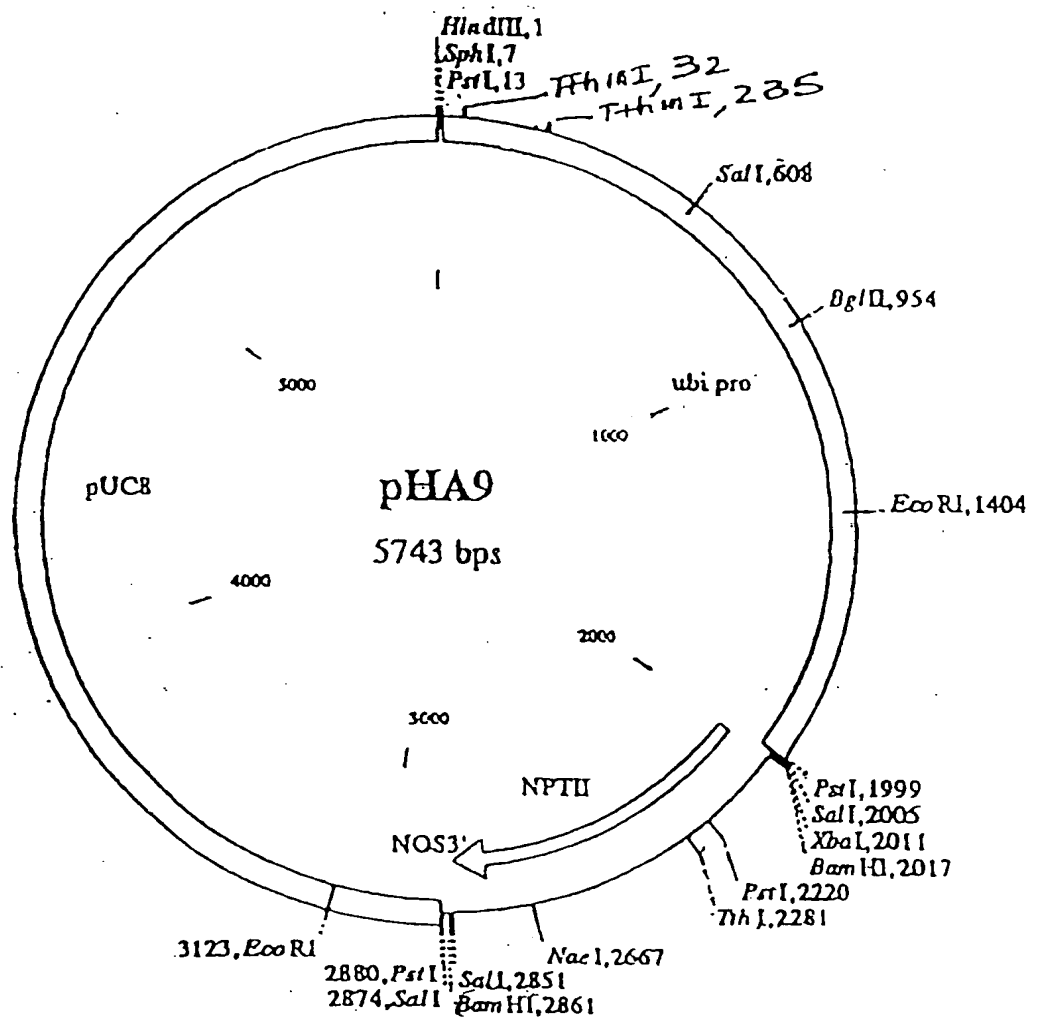
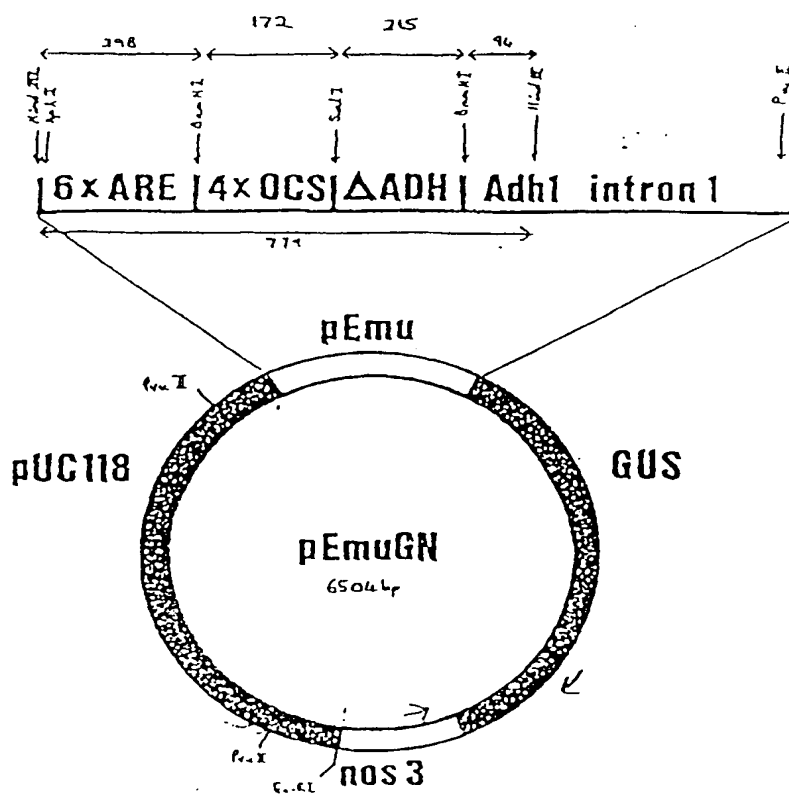


Figure 6



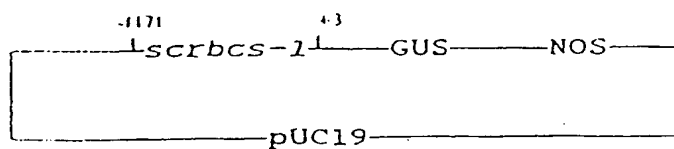


Figure 7

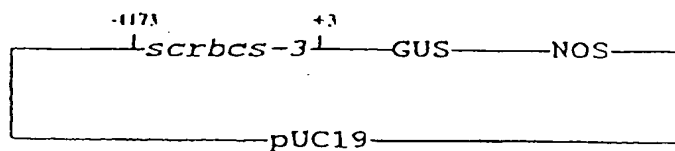


Figure 8

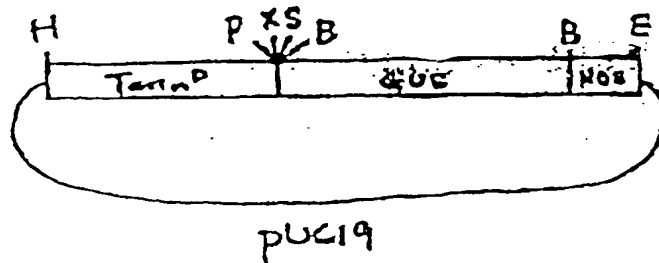


Figure 9

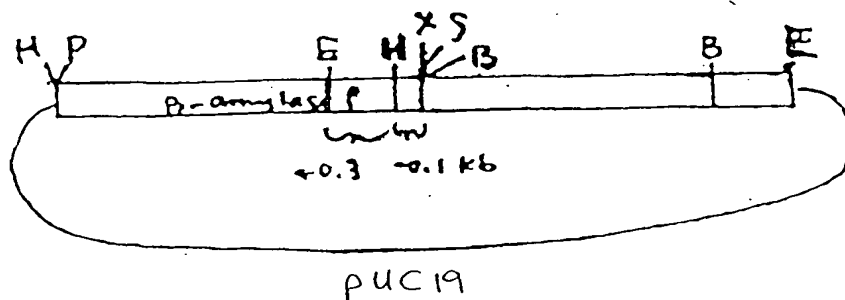


Figure 10

Figure 11

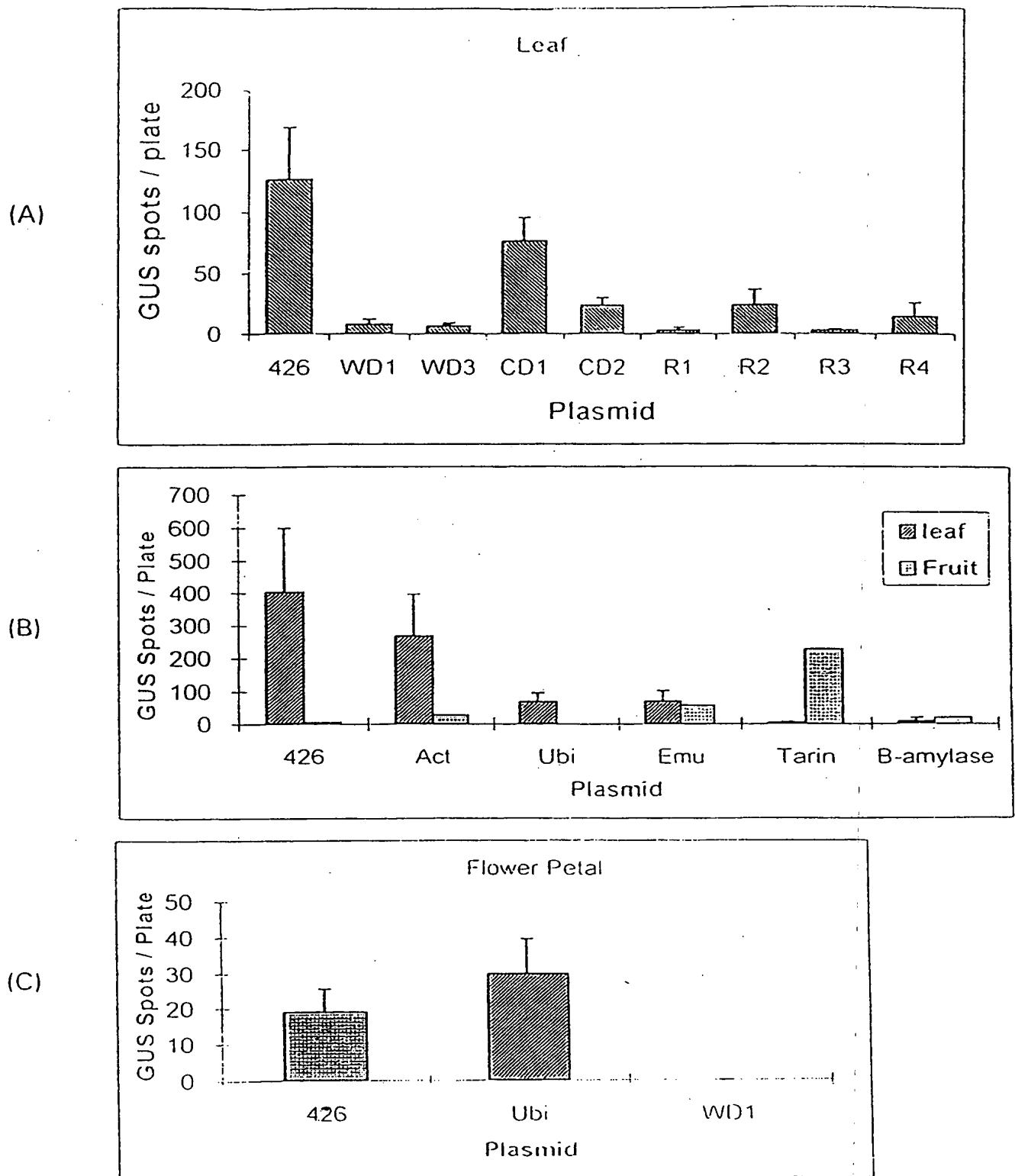


Figure 12

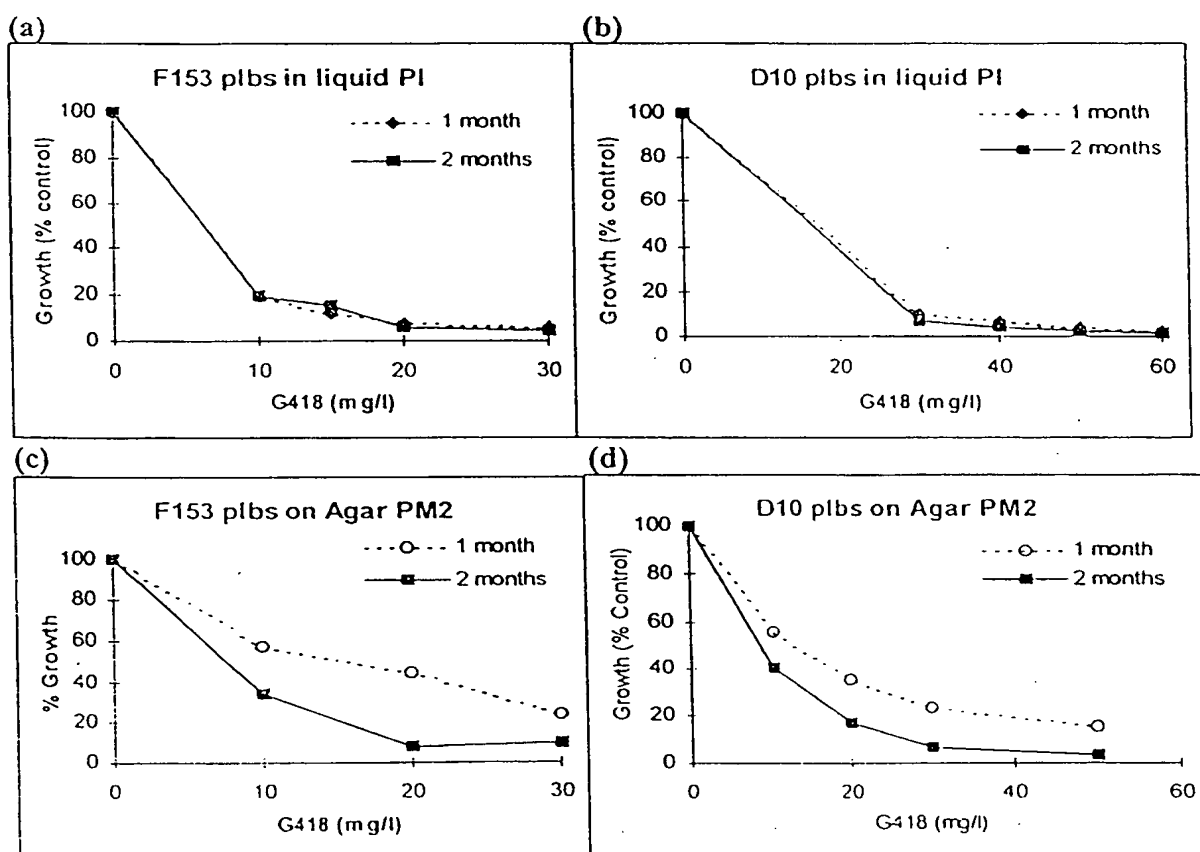


Figure 13

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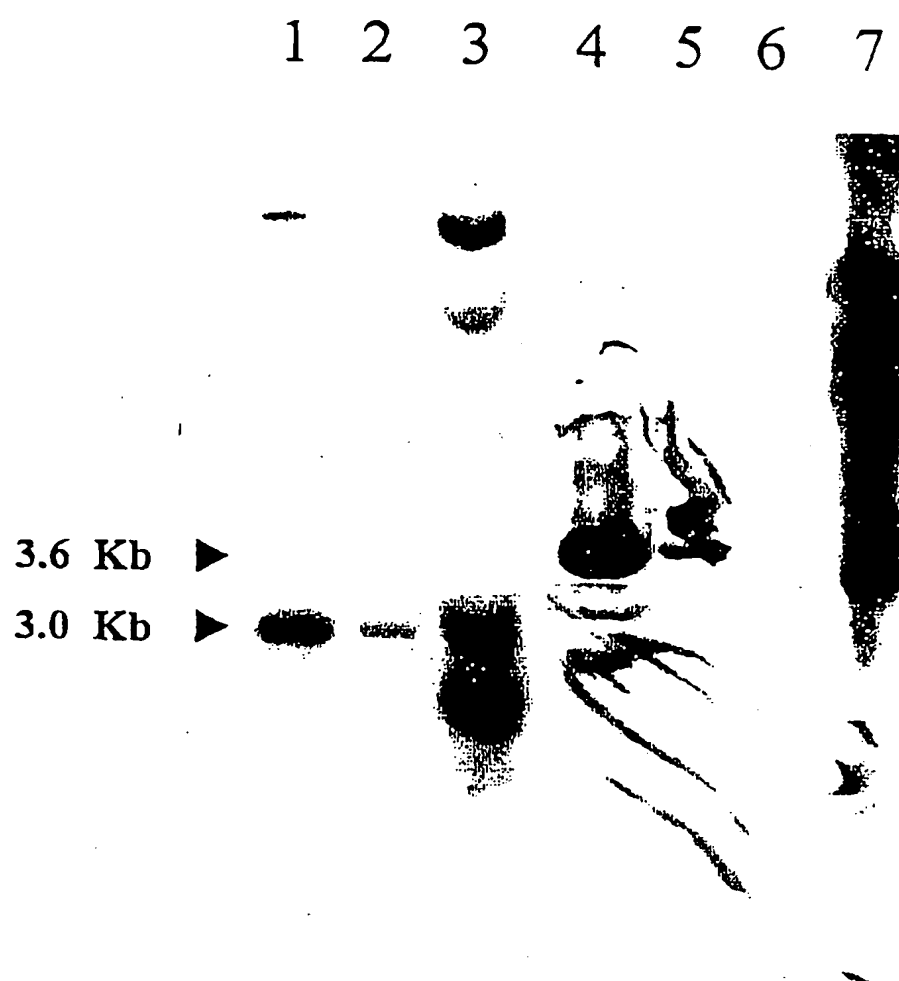
Figure 14

1 gaattccggc gtggcgctg ggctagtgt cccgcagcga gcgatctgag agaacggtag
 61 agttccggcc gggcgcgcg gagaggagga gggtcgggcg gggaggatcc gatggccggg
 121 aacgagtggg tcaatgggta cctggaggcg atcctcgaca gccacacctc gtcgcggggt
 181 gccggcgcg ggcgcggcg gggggacccc aggtcgccga cgaaggcggc gagccccgc
 241 ggcgcgcaca tgaacttcaa cccctcgacac tacttcgtcg aggagggtgt caagggcgtc
 301 gacgagagcg acctccaccg gacgtggatc aaggtcgtcg ccaccgcga cgcccgcgag
 361 cgcagcacca ggctcgagaa catgtgtg cggatctggc acctcgcgcg caagaagaag
 421 cagctggagc tggaggcat ccagagaatc tcggcaagaa ggaaggaaca ggagcaggtg
 481 cgtcgtgagg cgacggagga cctggccgag gatctgtcag aaggcgagaa gggagacacc
 541 atcgccgagc ttgcgccgtg tgagacgacc aagaagaagt tccagaggaa ctctctgac
 601 cttaccgtct ggtctgacga caataaggag aagaagcttt acattgtgt catcagcgtg
 661 catggtcttg ttcgtggaga aaacatggaa ctaggctgtg attctgatac aggtggccag
 721 gtgaaatatg tggcgaact tgcaagagcg atgtcaatga tgcctggagt gtacaggggtg
 781 gacctcttca ctgctcaagt gtcattcct gacgtggact ggagctacgg tgagccaacc
 841 gagatgttat gcgccgggtc caatgatgga gaggggatgg gtgagagtg cgagcctac
 901 attgtgcgca taccgtgtgg gccgcgggat aaatactca agaaggaagc gttgtggcct
 961 tactccaag agtttgcga tggagccctt gcgcataacc tgaacatgt caaggctctg
 1021 ggagagcagg ttggaatgg gaggccagta ctgccctacg tgatacatgg gcactatgcc
 1081 gatgtcgag atgttctgc tctctttct ggtgcgtga atgtccaat ggtgtcact
 1141 ggccactcac ttgggaggaa caagctggaa caactgctga agcaaggcg catgtccaag
 1201 gaggagatcg attcgacata caagatcatg aggcgtatcg agggtagga gctggccctg
 1261 gatgcgtcag agcttgaat cagagcaca aggcaggaga ttgatgagca gtggggattg
 1321 tacgatggat ttgatgtcaa gcttgagaaa gtgctgaggg cacgggcgag gcgcgggggt
 1381 agctgccatg gtcgttacat gcctaggatg gtggtgattc ctccgggaat ggattcagc
 1441 aatgtttag ttcataaga cattgatggg gatggtgacg tcaaagatga tatgttgg
 1501 ttggagggtg cctcaccaa gtcaatgcc ccaatttggg ccgaagtga gcggttctg
 1561 accaaccctc acaagccgat gatcctggcg ttatcaagac cagaccgaa gaagaacatc
 1621 actaccctcg tcaaagcgtt tggagagtgt cgtccactca gggaaactgc aaaccttact
 1681 ctgatcatgg gtaacagaga tgacatcgac gacatgtctg ctggcaatgc cagtgtctc
 1741 accacagttc tgaagctgat tgacaaglat gatctgtacg gaagcgtggc gttccctaag
 1801 catcacaatc aggtgacgt cccggagatc tatgcctcg cggccaaaat gaaggcgctc
 1861 ttcatcaacc ctgctctgt tgagccgtt ggtctcacc tgatcgaggc tgcggcacac
 1921 ggactcccga tagtcgtac caagaatgtt ggtccggtcg acattacaa tgcattaaac
 1981 aacggactgc tctgtgacct acacgaccag aacgccatcg ctgatgcact gctgaagctt
 2041 gtggcagaca agaaccgtg gcaggaatgc cggagaaacg ggttcgcaa catccacctc
 2101 tactcatggc cggagcactg ccgcacttac ctaccaggg tggccgggtg ccggttaagg
 2161 aaccgaggt ggtgaagga cacaccagca gatgccggag ccgatgagga ggagttcctg
 2221 gaggattcca tggacgtca ggacctgtca ctccgtctgt ccacgacgg tgagaagagc
 2281 tcgtgaaca ctaacgatcc actgtgttgc gaccccgagg atcaagtga gaagatcatg
 2341 aacaacatca agcagtcgtc agcgttctt ccgtccatgt cctcagtcgc agccgagggc

Figure 14 (Contd.)

2401 acaggcagca ccatgaacaa ataccacac ctcgcgcggc gccggcgctt gttcgtcata
2461 gctgtggact gctaccagga ccatggccgt gctagcaaga agatgctgca ggtgatccag
2521 gaagttttca gaggagtcg atcggactcc cagatgttca agatctcagg gttcagctg
2581 tcgactgcca tggcgttgc cagacacac cagctctgc agctcggcaa gatccagcg
2641 accgacttcg acgccctcat ctgtggcagc ggcagcgagg tctactatcc tggcacggcg
2701 aactgcatgg acgtgaagg aaagctgcgc ccagatcagg actatctgat gcacatcagc
2761 caccgctggt cccatgacgg cgcgaggcag accatagcga agctcatggg cgtcaggac
2821 gggtcaggcg acgtgtcga gcaggacgtg gcgtccagta atgcacactg tctcgcgttc
2881 ctcatcaaag acccccaaaa ggtgaaaacg gtcgatgaga tgaggagcg gctgaggatg
2941 cgtggtctcc gctgccacat catgtactgc aggaactcga caaggcttca ggtgtccct
3001 ctgctagcat caaggtcaca ggcactcagg tatctttccg tgcgctgggg cgtatctgtg
3061 gggaacatgt atctgacac cggggaacat ggcgacaccg atctagagga gatgctatcc
3121 gggctacaca agaccgtgat cgtccgtggc gtcaccgaga agggttcgga agcactggtg
3181 aggagcccag gaagctacaa gagggacgat gtcgtcccg ctgagacccc ctggtctgcg
3241 tacacgactg gtgagctgaa ggccgacgag atcatgcggg ctctgaagca agtctccaag
3301 acttcagcg gcatgtgaat ttgatgttc ttltacatt tctctttc ttactgcta
3361 tataaaataa gttgtgaaca gtaccgaggg tgtgtatata tatattgcag tgacaaataa
3421 aacaggacac tgctaactat actgggtgaat atacgactgt caagattgta tgctaagtac
3481 tccatttctc aatgtatcaa tcggaattc

Figure 15



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10576

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 5/04, 15/63, 15/64, 15/82, 15/84; A01H 1/00, 1/04, 5/00

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Agricola, Caplus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PENA et al. Agrobacterium-mediated Transformation Of Sweet Orange And Regeneration Of Transgenic Plants. Plant Cell Rep. 1995, Vol. 14, pages 616-619, especially pages 616-617, see entire document.	19-23, 26
Y	McCABE et al. Direct DNA Transfer Using Electric Discharge Particle Acceleration (ACCELL Technology). Plant Cell Tiss. Org. Cult. 1993, Vol. 33, pages 227-236, especially pages 227-228, 231-233.	1-18, 24, 25, 27-32
Y	GAMBORG, O.L. Plant Cell Cultures: Nutrition And Media. in Cell Culture and Somatic Cell Genetics of Plants. 1984, Vol. 1, pages 18-26, especially pages 18-19.	1-32

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*g* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

01 JULY 1999

Date of mailing of the international search report

10 SEP 1999

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10576

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MAPES, M.O. Tissue Culture Of Bromeliads. The International Plant Propagators' Society Combined Proceedings. 1973, Vol. 23, pages 47-55, especially pages 48-50, see entire document.	2-4, 7-14, 17-27, 30-35
Y	CHIA et al. The Firefly Luciferase Gene As A Non-invasive Reporter For Dendrobium Transformation. Plant J. 1994, Vol. 6, No. 3, pages 441-446 especiall page 446, see entire document.	18, 25
Y	RANGAN, T.S. Pineapple. In Handbook of Plant Cell Culture. P. K. Ammirato et al, eds. 1984, pages 373-382, especially pages 374-375, 379.	1-32
Y	ZHU et al. Isolation Of Genomic DNAs from Plants, Fungi And Bacteria Using Benzyl Chloride. Nucl. Acids Res. 1993, Vol. 21, No. 22, pages 5279-5280, see whole document.	16, 29
Y	SABELLI et al. Nucleic Acid Blotting And Hybridisation. Meth. Plant Biochem. 1993, Vol. 10, pages 79-100, see whole document.	16, 29
Y	WITTY, M. Thaumatin II: A Sweet Marker Gene for Use In Plants. Meth. Enzymol. 1992, Vol. 216, pages 441-447, especially pages 442-443.	31
Y	HAMILTON et al. Antisense Gene That Inhibits Synthesis Of The Hormone Ethylene In Transgenic Plants. Nature. 19 July 1990, Vol. 346, pages 284-287, see whole document.	32

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10576

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.
No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10576

A. CLASSIFICATION OF SUBJECT MATTER: US CL :

435/69.1, 410, 418, 419, 420, 430, 431, 430.1, 468, 469, 470; 800/278, 279, 283, 285, 286, 288, 293, 294, 295, 298, 301, 302

B. FIELDS SEARCHED

Minimum documentation searched
Classification System: U.S.

435/69.1, 410, 418, 419, 420, 430, 431, 430.1, 468, 469, 470; 800/278, 279, 283, 285, 286, 288, 293, 294, 295, 298, 301, 302

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-18, 24, 25, 27-32, drawn to a first product, a stably transformed transgenic pineapple-like totipotent body; first method of transforming a pineapple-like totipotent body via particle bombardment.

Group II, claim(s) 19-23, 26, drawn to a second method of transforming a pineapple-like totipotent body, via Agrobacterium.

Group III, claim(s) 33-35, drawn to a third method, for producing a pineapple-like totipotent body.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The special technical feature of plant transformation via particle bombardment is not shared nor required by the other groups. The special technical feature of plant transformation via Agrobacterium is not shared nor required by the other groups. The invention of group three does not share nor require the transformation methods of the other groups.